Reduction of Superoxide Dismutase Activity Correlates with Visualization of Edema by T₂-weighted MR Imaging in Focal Ischemic Rat Brain

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Abstract

This study investigated the correlation between *in vivo* serial T₂-weighted magnetic resonance (MR) imaging and changes in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, and water, sodium ion (Na⁺), and potassium ion (K⁺) contents measured *in vitro* using rat brain following right middle cerebral artery occlusion in conjunction with bilateral common carotid artery (CCA) occlusion. One hour later the left CCA was released. Serial MR images showed edema developed from the outer cortex towards the center. The T₂ signal intensity of the injured right cortex increased with time compared to that of the contralateral cortex. Increased Na⁺ and water and decreased K⁺ contents occurred in the injured cortex, indicating that serial T₂-weighted MR imaging reflects the changes in water content and Na⁺ and K⁺ concentrations determined by biochemical techniques. GSH-Px activity was little changed. Total SOD in the injured cortex decreased 1 hour after ischemia and remained low throughout the experiment. In contrast, SOD activity in the noninfarcted left cortex also decreased after 1 hour but returned to normal after 2 hours of ischemia. Our results suggest that oxygen free radicals are important in developing ischemic brain edema and cerebral infarction.

Key words: magnetic resonance imaging, focal ischemia, middle cerebral artery occlusion, superoxide dismutase, glutathione peroxidase

Introduction

Several magnetic resonance (MR) imaging studies have investigated the development of focal brain edema caused by ischemic insult in monkey,³¹⁾ feline,^{5,6)} and rat^{2,9,34)} middle cerebral artery (MCA) occlusion models. These studies successfully demonstrated cerebral edema after induction of ischemia, most consistently on the T₂-weighted images, but lacked specificity in the ischemic focus due to the occlusion procedure,⁵⁾ or resolution because of the small size of the rat brain.³⁴⁾

Biochemical studies have investigated the contents of sodium (Na⁺) and potassium ions (K⁺), and water

in ischemic brain, but the focal brain ischemia models used have also lacked specificity in the focus location.^{4,38,41)} The involvement of oxygen free radicals in ischemic brain injury has also been widely studied in various animal models,^{8,13,15,19,21,23,34,37,40)} but there is little information on the levels of endogenous antioxidation enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px).^{12,29)} None of these studies were associated with an MR imaging study.

A rat focal cerebral ischemia model using distal MCA and bilateral common carotid artery (CCA) occlusion with reperfusion of the left CCA after 1 hour provides cortical infarcts with reproducible size and

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location only in the ischemic MCA territory.¹⁶⁾ This study used this rat ischemia model to investigate the *in vivo* serial T₂-weighted MR images, taken using a special imaging coil,¹⁾ and associated changes in SOD and GSH-Px activities, Na⁺ and K⁺ concentrations, and water content, in a localized cortical infarct focus.

Materials and Methods

I. Animal preparation

Adult male Sprague-Dawley rats weighing 300–400 g were anesthetized with an intraperitoneal injection of 100 mg/kg chloral hydrate. A catheter was placed in the femoral artery to monitor blood pressure and obtain the blood samples for gas and pH analysis. Body temperature was maintained at 37°C with a heating pad controlled by a rectal probe. Blood pressure was maintained above 90 mmHg and blood gases were analyzed twice during the operation.

Focal cerebral ischemia was induced according to the method of Chen *et al.*¹⁶⁾ with slight modifications based on the anatomical variation of the MCA in rats.^{4,32)} Briefly, the right MCA was exposed for 1–2 mm under the junction of the zygomatic bone and skull to locate the pyriform branch. The right MCA was ligated proximal to the pyriform branch with a single loop of 10-0 suture. This procedure reduces the blood flow of the MCA to facilitate coagulation using a low-power electroprobe. The bilateral CCAs were immediately carefully exposed to avoid damage to the vagal nerve. The right CCA was ligated and the left CCA was occluded for 1 hour using a Scoville-Lewis microclip (4.0 × 0.7 mm) for rats used in the *in vitro* biochemical study. Occlusion of the CCAs and recirculation of the left CCA were controlled by elastic tubes looped around the CCAs and sternocleidomastoid muscles for rats used in the MR imaging study.

II. MR imaging study

Proton MR imaging used a Quest 4300 imaging spectrometer (Nalorac Cryogenics Corp., Martinez, Cal., U.S.A.) connected to a horizontal bore 4.7-Tesla Oxford magnet (usable bore 22.5 cm in diameter). A 3-inch diameter slotted-tube resonator, tuned to the proton frequency at 200.11 MHz, was used as an imaging coil.¹⁾ Two scans were acquired using a selective 90°-selective 180° spin-echo imaging pulse sequence for each of the 128 phase-encoding steps. The final image data matrix was 256 × 256. T₂-weighted imaging used repetition/echo times 4500/80 msec, 3 mm slice thickness, field

of view 80 × 80 mm, and a total acquisition time of 20 minutes.

After surgical MCA occlusion, the anesthetized rat was secured in a plastic cradle that was placed in the imaging coil. Preischemia images were first obtained. The bilateral CCAs were then occluded by pulling the elastic tubes while the rat was inside the magnet. Images were obtained after 1 hour. The left CCA was then released from the loop. T₂-weighted images were then obtained at 2, 4, 8, and 24 hours after bilateral CCA occlusion. A total of six rats survived the complete experiment.

The image data were transferred to a Sun Sparc Station (Mountain View, Cal., U.S.A.) for image display and region-of-interest (ROI) data analysis using a modified image tool program (National Center for Supercomputing Applications, San Francisco, Cal., U.S.A.). Image pixel intensity of the ROI was obtained in the injured right cortex and noninjured contralateral cortex. The signal intensity ratio was defined as the ratio of the mean ROI signal intensity of an injured region to that of the contralateral region.

III. SOD and GSH-Px activities

Enzyme activities were measured preischemia and 1 hour (before release of the left CCA clip), 2, 8, and 24 hours after bilateral CCA occlusion in the injured and contralateral cortices. The MCA and CCAs were only exposed in the preischemia and 24-hour sham-operated rats. Each group consisted of five animals, except the 24-hour ischemia group which contained 10 animals.

Samples of brain tissue in ischemic rats were obtained from two regions, the injured right cortex and the corresponding left cortex. Preischemia and sham-operated rat brain samples were dissected from the cortex just posterior to the MCA trunk. Animals were anesthetized with 35 mg/kg pentobarbital intraperitoneal injection prior to sacrifice. The brains were perfused *via* the ascending aorta with 250 ml of 0.9% saline to eliminate blood from the brain prior to removal. The cortex was quickly removed, weighed (80–150 mg wet weight was removed), and the samples homogenized in 2 ml 50 mM potassium phosphate buffer (pH 7.8) in the presence of 1 mM ethylenediaminetetra-acetic acid (EDTA) and then centrifuged at 4000 g for 20 minutes. The supernatants were stored at –70°C until enzyme assay, performed within 3 days.

SOD activity was assayed by inhibition of superoxide anion-dependent reactions.^{17,27,28)} The assay medium contained 30 M cytochrome c, 150 μmol xanthine, and brain homogenate from different brain

regions in 50 mM phosphate buffer (pH 7.8), to a final volume of 3 ml. The reaction was initiated by adding 10 μ l of xanthine oxidase (1.0 U/mg protein), and the absorbance at 550 nm was recorded for 5 minutes. One unit of SOD was defined as the amount of SOD in brain homogenate required to inhibit the rate of cytochrome c reduction by 50%. To assay total SOD activity, 10 μ M potassium cyanide (KCN) was added to the incubation medium to inhibit cytochrome c oxidase activity. To assay Mn-SOD activity, 1 mM KCN was added to the incubation mixture to inhibit CuZn-SOD activity. The activity of CuZn-SOD was derived by subtraction of Mn-SOD activity from the total SOD activity.^{12,15,23)}

GSH-Px was assayed in medium containing 0.1 ml brain homogenate, 0.1 ml 2.4 U GSH reductase (Sigma, St. Louis, Mo., U.S.A.), 0.1 ml 10 mM reduced GSH, and 0.5 ml 0.1 mM potassium phosphate buffer (pH 7.0), with 1 mM EDTA and 2 mM sodium azide to inhibit catalase activity. The reaction was initiated by adding 0.1 ml of 1.5 mM nicotinamide adenine dinucleotide phosphate (NADPH) (for 3 min) followed by 0.1 ml 1.5 mM H₂O₂ (for 5 min). The rate of disappearance of the NADPH absorbance at 340 nm was followed spectrophotometrically. One unit of GSH-Px was defined as the amount of enzyme that decomposed 1 nM NADPH/min at pH 7.0 at 25°C.^{12,24)}

Both SOD and GSH-Px activities are expressed as U/mg protein. Protein was assayed based on the methods of Lowry *et al.*²⁶⁾

IV. Water and ion concentrations

Water and ion concentrations were measured at the same times as the enzyme activity. The preischemia group contained 10 animals, the 24-hour ischemia group 20 animals, and all other groups five animals for water content determination. For Na⁺ and K⁺ content determination, each group contained five animals except the 24-hour ischemia group which contained 20 animals.

Tissue (35–80 mg wet weight) was dissected and dried in a desiccating oven at 105°C for 24 hours, and reweighed to obtain the dry weight. Water contents were expressed as a percentage of wet tissue weight.^{14,41)} To prepare tissue samples for atomic absorption spectroscopic measurement of Na⁺ and K⁺, the dried samples were first digested in 5 ml of 1 N nitric acid for 5 days. A 0.1 ml aliquot was diluted with 9.9 ml of deionized water. The Na⁺ and K⁺ concentrations were then measured using this solution by atomic absorption spectroscopy at 589.0 and 766.5 nm, using a hollow Na⁺, K⁺ cathode lamp, a

premix burner, and an acetylene-air flame. The measurement was bracketed with two standards, one containing only the diluent and the other including diluent plus a known concentration of Na⁺ or K⁺.⁴¹⁾

V. 2,3,5-Triphenyltetrazolium chloride (TTC) staining

The 20 animals used for Na⁺ and K⁺ determination at 24 hours were also used for TTC staining.

Staining used the immersion technique, with 2% TTC solution in Dulbecco's phosphate buffer. At 24 hours after ischemia, the brains were removed immediately without perfusion.^{3,4)} Brain tissue at the site of the MCA trunk was cut into 2 mm-thick slices using a brain slicer. The slices were covered with cover slips smeared with the TTC solution to prevent distortion and to amplify staining on the brain surface in contact with the 2% TTC solution, and kept at 40°C for 20 minutes. The brain slices were then placed in 10% buffered formalin and stored in the dark in a refrigerator until photographed. The rostral surface of the brain slices was photographed using color slide film (ASA64-Daylight, EPR 135-36; Kodak Ektachrome, Rochester, N.Y., U.S.A.). Tracings of the slides of TTC-stained brain slices were made. The infarcted area was quantified by cutting and weighing the traced sections of normal and infarcted areas. The area of infarction was expressed as a percentage of the whole coronal section.

VI. Data analysis

Data were analyzed using Student's t-test or by analysis of variance followed by multiple range test. A p value of 0.05 or less was considered significant.

Results

The relative intensity of the injured cortex on the T₂-weighted MR images increased continuously with time from 99.87% of the preischemic value to 206.95% 24 hours after ischemia (Table 1). Edema began to form 2 hours after bilateral CCA occlusion. Cortical edema was distinctive 4 hours after ischemia. A ring-like high-intensity area in the outer zone of the injured cortex appeared after 8 hours. Brain images 24 hours after ischemia clearly showed remarkably enhanced cortical edema. A typical case is shown in Figs. 1 and 2.

The total SOD (CuZn-SOD + Mn-SOD) levels in the injured and contralateral cortices of preischemic animals were similar (Table 2). The total SOD levels in the injured cortex were significantly decreased dur-

Table 1 MR signal intensity, water content, and Na⁺ and K⁺ concentrations in the injured and contralateral cortices

	MR intensity (%)	Water (%)	Na ⁺ (mEq/kg d.w.)	K ⁺ (mEq/kg d.w.)
	Injured/contralateral	Injured/contralateral	Injured/contralateral	Injured/contralateral
Preischemia	99.87 ± 2.2 /100.00 (n = 6)	78.89 ± 0.14 /78.97 ± 0.10 (n = 10)	342 ± 4 /340 ± 15 (n = 5)	502 ± 7 /492 ± 2 (n = 5)
Postischemia				
1 hr	105.74 ± 4.64 /100.00 (n = 6)	81.06 ± 0.47 /79.06 ± 0.20 (n = 5)	383 ± 23 /320 ± 16 (n = 5)	506 ± 10 /514 ± 10 (n = 5)
2 hrs	122.70 ± 10.11**/100.00 (n = 6)	81.51 ± 0.10*/79.22 ± 0.66 (n = 5)	406 ± 9**/329 ± 12 (n = 5)	334 ± 2*/515 ± 11 (n = 5)
4 hrs	131.79 ± 6.65* /100.00 (n = 6)	—	—	—
8 hrs	152.10 ± 6.44* /100.00 (n = 6)	84.61 ± 0.28*/78.82 ± 0.35 (n = 5)	743 ± 58* /332 ± 11 (n = 5)	209 ± 10*/519 ± 12 (n = 5)
24 hrs	206.95 ± 13.80* /100.00 (n = 6)	86.98 ± 0.41*/78.23 ± 0.21 (n = 20)	1102 ± 40* /338 ± 7 (n = 20)	152 ± 8*/490 ± 8 (n = 20)
24-hr sham-operated	—	78.82 ± 0.31 /78.66 ± 0.22 (n = 5)	343 ± 5 /339 ± 9 (n = 5)	498 ± 5 /489 ± 6 (n = 5)

Values are expressed as mean ± SEM. Significant difference from preischemia and contralateral values given as *p < 0.005 and **p < 0.05. T₂-weighted MR signal intensity of the infarcted cortex as a percentage of the signal intensity in the contralateral cortex, showing an increase with time. Water content and Na⁺ and K⁺ concentrations changed gradually in the injured cortex. The T₂ signal intensity *in vivo* and water and ion concentrations *in vitro* are correlated.

Fig. 1 Serial T₂-weighted MR images of focal ischemic rat brain, 0, 1, 2 (upper left to right), 4, 8, and 24 hours (lower left to right) after ischemia, showing edema in the right MCA cortical territory developing from 2 hours. Cortical edema develops from the outer zone of the MCA cortex to the center. Images at 24 hours after ischemia clearly showed the most intense edema.

ing ischemia (*i.e.* MCA occlusion + bilateral CCA occlusion) and remained low at 2, 8, and 24 hours following reperfusion of the left CCA. At 24 hours following ischemia, the total SOD activity was reduced by 61% in the injured cortex. The total SOD activity was transiently reduced in the contralateral cortex during ischemia and returned to the preischemic levels thereafter. The levels of total SOD in

the 24-hour sham-operated animals were unchanged from the preischemic values. Similarly, the activities of CuZn-SOD and Mn-SOD in the injured cortex were significantly reduced following ischemic insult, and remained lower than the preischemic values after reperfusion of the left CCA, while reversible changes in enzyme activity were observed in the contralateral cortex. In the 24-hour group, CuZn-

Table 2 Change in antioxidative enzyme activity in the injured and contralateral cortices

	Total SOD (U/mg protein)	CuZn-SOD (U/mg protein)	Mn-SOD (U/mg protein)	GSH-Px (U/mg protein)
	Injured/contralateral	Injured/contralateral	Injured/contralateral	Injured/contralateral
Preischemia	5.03 ± 0.29 / 5.04 ± 0.24 (n = 5)	4.27 ± 0.33 / 4.27 ± 0.23 (n = 5)	0.77 ± 0.07 / 0.77 ± 0.08 (n = 5)	14.26 ± 0.31 / 14.89 ± 0.25 (n = 5)
Postischemia				
1 hr	1.78 ± 0.08* / 1.36 ± 0.29* (n = 5)	1.62 ± 0.13* / 1.34 ± 0.28* (n = 5)	0.16 ± 0.07* / 0.02 ± 0.02* (n = 5)	14.50 ± 0.23 / 14.70 ± 0.43 (n = 5)
2 hrs	1.88 ± 0.06* / 3.98 ± 0.20* (n = 5)	1.85 ± 0.04* / 3.55 ± 0.16 (n = 5)	0.03 ± 0.03* / 0.44 ± 0.09 (n = 5)	15.20 ± 0.19** / 14.96 ± 0.62 (n = 5)
8 hrs	1.92 ± 0.06* / 4.64 ± 0.25 (n = 5)	1.60 ± 0.06* / 3.89 ± 0.16 (n = 5)	0.33 ± 0.08* / 0.75 ± 0.11 (n = 5)	15.07 ± 0.46 / 14.77 ± 0.54 (n = 5)
24 hrs	1.94 ± 0.10* / 5.06 ± 0.16 (n = 10)	1.30 ± 0.11* / 4.08 ± 0.15 (n = 10)	0.64 ± 0.07 / 0.98 ± 0.06 (n = 10)	16.05 ± 0.89* / 14.65 ± 0.69 (n = 10)
24-hr sham-operated	4.99 ± 0.23 / 4.26 ± 0.27 (n = 5)	4.34 ± 0.13 / 3.48 ± 0.25 (n = 5)	0.65 ± 0.13 / 0.78 ± 0.03 (n = 5)	15.08 ± 0.61 / 15.50 ± 0.79 (n = 5)

Values are expressed as mean ± SEM. Significant differences from preischemic values given as * $p < 0.005$ and ** $p < 0.05$. Total SOD in the injured cortex decreased 1 hour after ischemia and remained low throughout the experiment. SOD activity in the contralateral cortex also decreased at 1 hour but returned to normal after reperfusion. No reduction in GSH-Px activity was found.

Fig. 2 T₂-weighted MR images after ischemia, showing ring-like cortical edema in the 8-hour image (left) and enhanced edema in the 24-hour image (right).

Fig. 3 Typical TTC staining of a brain slice from the focal ischemia model, revealing the infarcted region as an unstained area. The infarction is similar to the T₂-weighted MR images shown in Figs. 1 and 2.

SOD activity was reduced by 70% in the injured cortex. The Mn-SOD activity was reduced by 79, 96, 57, and 17% at 1, 2, 8, and 24 hours in the injured cortex.

GSH-Px activity did not change except 24 hours postischemia in the injured cortex (Table 2). A slight increase in GSH-Px activity occurred in the infarcted focus. No changes in GSH-Px activity were observed in the contralateral cortex.

The water content in the injured cortex increased with time following ischemia (Table 1). No changes in water content were seen in the contralateral cortex or the 24-hour sham-operated group.

The Na⁺ concentration in the injured cortex increased with time following ischemia (Table 1). No changes in Na⁺ concentration were observed in the contralateral cortex or the 24-hour sham-operated group. The K⁺ concentration in the injured cortex

decreased with time following ischemia. No changes in K⁺ concentration were observed in the contralateral cortex or 24-hour sham-operated group.

TTC staining demonstrated the infarcted region as an unstained area in all cases. The percentage of infarction was $14.45 \pm 2.52\%$ (mean ± SD). A typical slice sample is presented in Fig. 3.

Discussion

Like the previous MR imaging studies conducted in rats,^{2,9,34} felines,⁵⁻⁷ or monkeys,³¹ the present MR imaging study revealed distinctive cerebral edema within 120 minutes of induction. Our study also demonstrated a positive correlation between the change in T₂-relative intensity and increase in water

content and Na^+ concentration, and decrease in K^+ concentration (Table 1). These data indicate that serial T_2 -weighted MR images of ischemic rat brain reflect the changes in water content and Na^+ and K^+ concentrations as determined by biochemical techniques *in vitro*. The progress of cortical edema formation was interesting. Water accumulation spread from the outer zone to the center of the MCA cortical territory on the serial T_2 -weighted images (Figs. 1 and 2). Possibly the oxygen supply in the border area is relatively rich compared to the central area so that oxygen-derived free radicals are generated and propagate to damage the blood-brain barrier,^{22,39)} resulting in edema formation beginning from the outer zone.

Previous studies using Tamura's MCA occlusion rat model^{30,38,41)} showed that water content in a MCA area resembling our sampling territory increased 1.9 fold after 24 hours of ischemia. The Na^+ concentration increased 4.3 fold, and the K^+ concentration decreased to 34% after 24 hours of ischemia. Our study found that the water content in the MCA cortex increased 1.1 fold after 24 hours of ischemia, while the Na^+ concentration increased 3.2 fold, and the K^+ concentration decreased to 30% (Table 1). The greater change in water and Na^+ contents in Tamura's focal brain ischemia model may be due to injury of not only MCA cortex but also the MCA subcortex and basal ganglia. Expanded coagulation of the MCA trunk between the olfactory tract and the inferior cerebral vein to avoid variation and multiplicity of infarcted focus⁴⁾ in Tamura's focal ischemia mode has been recommended, but such a complex surgical procedure for experimental animals may damage the brain during the long operation.

SOD, a widely distributed and highly conserved enzyme in various cells, is the first line of defense against the deleterious effects of oxygen-derived free radicals.^{18,36)} In brain tissue, CuZn-SOD occurs in the cytoplasm and Mn-SOD in mitochondria. These enzymes convert superoxide anion (O_2^-) to hydrogen peroxide which is decomposed into water and oxygen by GSH-Px and/or catalase. Reduced SOD activity will compromise the central nervous system (CNS) defense against superoxide radical-induced oxidative injury.^{35,39)} Supplementation with exogenous SOD protects cultured neurons from death by starvation³³⁾ and ameliorates arachidonic acid-induced astrocytic swelling.¹⁰⁾ Although endogenous SOD levels were not measured in these studies, an oxidative imbalance during injury is clearly associated with exogenous SOD, indicating that a high level of SOD is beneficial in maintaining the normal physiological

and biochemical function of the CNS. In contrast, Michowiz *et al.*²⁹⁾ reported no significant change in SOD activity relative to the control value in any brain area up to 24 hours in their own MCA occlusion rat model, concluding that endogenous SOD is not important in the acute phase of cerebral ischemia.

Our results demonstrated a significant decrease in CuZn-SOD and Mn-SOD activities in the contralateral left cortex following right MCA and bilateral CCA occlusion for 1 hour. These data suggest that SOD concentration was sensitive to ischemic insult, even in the left cortex which only underwent temporary CCA occlusion. The biochemical mechanism underlying reduced SOD activity in the left cortical region is not clear at present, and requires further elucidation. Nevertheless, these results generally agree with our previous report that SOD activity decreased in the brain cortex following complete ischemia produced by decapitation.¹²⁾ Reperfusion of the left CCA after 1 hour caused a return to the preischemic levels of both CuZn-SOD and Mn-SOD in the noninjured left MCA territory, while SOD activity in the injured right MCA territory remained low, suggesting that oxygen and/or other factors, such as enzyme activators or inhibitors, are required during reperfusion for expression of SOD activity. Another possibility is that enzyme synthesis may increase during postischemic reperfusion. Studies of messenger ribonucleic acid levels of SOD are needed to investigate this possibility. The reduction in Mn-SOD activity in the injured MCA cortical territory was similar to that for CuZn-SOD, suggesting that mitochondrial enzyme activities were also sensitive to oxygen deprivation during ischemia. However, Mn-SOD activity had returned to normal levels in the injured MCA cortical territory by 24 hours after ischemia (Table 2). The reason for this is unclear at present. One possible explanation may be the method of measurement. Since Mn-SOD values were determined by subtraction of CuZn-SOD from the total SOD activity, even a small decrease in CuZn-SOD (e.g. at 24 hrs) reflects a proportionally larger increase in Mn-SOD. Therefore the total SOD activity may be a better indication of the oxidative state in various brain regions following ischemia and reperfusion injury.

Our study also tried to identify any causal relationship between the early reduction in SOD activity and subsequent brain edema formation. This is still unclear, since the levels of SOD in both the injured and contralateral cortices were significantly reduced following the occlusion of the MCA and CCAs, but only the MCA-occluded cortical territory developed

an infarction. However, the decrease in SOD levels in the contralateral cortex was reversible, so infarction may depend on the period during which active free radicals are present when generated by the ischemic insult. The reversal of the fall in SOD levels 1 hour after occlusion in the contralateral cortex may have prevented free radicals injuring the brain tissue permanently. The time period of reduced SOD activity is therefore an important factor in causing irreversible infarction in brain tissue. Another possibility is that injurious biochemical factors other than the reduced SOD level operate in the MCA cortical territory, probably due to the higher level of residual regional cerebral blood flow (rCBF) and short duration of ischemia resulting in less free radical formation than in the infarcted cortex. Studies of rCBF and other biochemical factors in both the MCA-occluded cortical territory and corresponding contralateral cortex may provide useful information.

The effect of increased levels of exogenous cerebral SOD on cellular necrosis has been tested in the same focal ischemia model as in this study, showing that pretreatment with polyethylene glycol-conjugated SOD (PEG-SOD) and PEG-catalase prior to MCA and CCA occlusion reduced the infarct volume significantly.²³⁾ Although the effect of PEG-SOD alone was not investigated, this study suggests that the imbalance in oxidative state in the CNS is one of the major factors in the pathogenesis of ischemic infarct. In contrast, Haun *et al.*²⁰⁾ reported that PEG-SOD failed to augment brain SOD activity in ischemic and nonischemic piglets, and concluded that intravenous PEG-SOD administration does not increase SOD activity in the brain despite ischemia and reperfusion. We performed a further study employing liposome-entrapped SOD which showed the extended half-life of trapped SOD ameliorated the infarcted focus using the present focal ischemic rat brain model.²³⁾ Recently we showed that neurotoxicity was reduced in transgenic mice overexpressing human CuZn-SOD, indicating that transgenic neurons containing two to three times the normal CuZn-SOD activity as the result of expression of the human CuZn-SOD transgene are protected against glutamate neurotoxicity *in vitro*.¹¹⁾

The SOD reduction prior to the detection of brain edema and cerebral infarction by T₂-weighted MR imaging suggests that there may be a period allowing therapeutic intervention using antioxidative agents. T₂-weighted MR imaging can demonstrate the beginning of ischemic edema, whereas free radical-mediated reaction in the brain cells becomes irreversible only after a certain period. Therefore, early ad-

ministration of antioxidative may be effective to prevent permanent ischemic neuronal damage.

GSH-Px, an enzyme which detoxifies H₂O₂ and lipid peroxides, occurs in both mitochondria and cytoplasm. Our data demonstrated that GSH-Px activity changed little within 24 hours of ischemia, only increasing slightly in the infarcted focus. No changes occurred in the contralateral cortex. These results agree with the findings that GSH-Px activity is stable following decapitation ischemia and a prolonged postmortem period.¹²⁾ The slightly increased or unaffected GSH-Px activity in the infarcted focus may be the first line of defense against hydrogen peroxide, a product catalyzed by SOD, and also against lipid peroxides. No reduction in GSH-Px activity during ischemia and reperfusion can help avoid accumulation of hydrogen peroxide which is a product of SOD-catalyzed reaction.

Our study showed that serial T₂-weighted MR images of ischemic rat brain reflect the changes in water content and Na⁺ and K⁺ concentrations determined by *in vitro* biochemical techniques. The visualization of ischemic brain edema by T₂-weighted MR imaging is correlated with an extended period of reduced SOD activity. No reduction in GSH-Px activity occurred during ischemia or reperfusion. Our results suggest that oxygen free radicals are crucial in developing ischemic brain edema and cerebral infarction.

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